

# Oviposition behavior

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Updated date: Jan 6, 2020

 An abbreviated version of this protocol was published in eLIFE in Oct 2019

Robust olfactory responses in the absence of odorant binding proteins

DOI: 10.7554/eLife.51040

## Detailed protocol

Please see below, a more detailed description is provided as *italic* in addition to the methods described in Materials and Methods section.

Oviposition assays were carried out in Petri dishes containing four quadrants (Dot Scientific, CAT # 557684). *This kind of dish was originally used for larval assay, flies can easily explore all quadrants.* Two quadrants contained 0.25% (w/v) agarose with 1% (w/v) sucrose, which was added to elicit oviposition. *A slight higher concentration was prepared as later linoleic acid - ethanol will be added.* One of the two quadrants contained a sample of linoleic acid, while the other was a control. *Two quadrants were left blank to load flies.* To better solubilize the linoleic acid sample, it was first pre-mixed in a 0.2% (v/v) solution of ethanol, which was then added to agarose that was at 55–58°C. After vigorous shaking, 5 ml of this agarose was introduced into a quadrant. The other quadrant contained agarose prepared in exactly the same way, but with 0.2% ethanol that contained no linoleic acid sample. *An advice here is, always switch the order of introducing two quadrants.*

10 newly eclosed females were cultured with three males in a vial for 5–6 days. *Flies were collected on daily basis.* Shortly before the assay, flies were immobilized on ice, and female flies were gently placed into the two quadrants that contained no agarose. *Male flies were discarded.* Ten females were maintained in the Petri dish for 20–24 hr in a dark room (25°C, 60% humidity). An oviposition preference index (OPI) was then calculated as follows:  $OPI = (\text{number of eggs on stimulus quadrant} - \text{number of eggs on control quadrant}) / (\text{total number of eggs})$ . A very small fraction of dishes contained fewer than 10 eggs and were excluded from the assay. Mutant and control flies were tested in parallel in all cases.

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Xiao, S. (2020). Oviposition behavior. Bio-protocol Preprint. [bio-protocol.org/prep182](https://bio-protocol.org/prep182).
2. Xiao, S., Sun, J. S. and Carlson, J. R. (2019). Robust olfactory responses in the absence of odorant binding proteins. eLIFE. DOI: [10.7554/eLife.51040](https://doi.org/10.7554/eLife.51040)

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